Synthesis of the constrained glutamate analogues (2*S*,1′*R*,2′*R*)- and (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclobutyl)glycines L-CBG-II and L-CBG-I by enzymatic transamination

Xin Gu, Mo Xian, Sophie Roy-Faure, Jean Bolte, David J. Aitken* and Thierry Gefflaut*

Laboratoire SEESIB-CNRS (UMR 6504), Département de Chimie, Université Blaise Pascal—Clermont-Ferrand II, 24, Avenue des Landais, 63177 Aubière cedex, France

Received 12 October 2005; revised 26 October 2005; accepted 28 October 2005
Available online 16 November 2005

Abstract—Optically pure *trans*-cyclobutane analogues of glutamic acid are prepared by highly selective enzymatic transamination of a single racemic *trans*-cyclobutane α-ketoglutaric acid derivative **5**, which is synthesized in five steps from maleic anhydride. (2*S*,1′*R*,2′*R*)- and (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclobutyl)glycines L-CBG-II and L-CBG-I are obtained using aspartate aminotransferase (AAT) and branched chain aminotransferase (BCAT), respectively.
© 2005 Elsevier Ltd. All rights reserved.

1. Introduction

L-Glutamic acid (Glu) is the major excitatory neurotransmitter in the central nervous system of vertebrates. It interacts with two families of receptors, namely ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs).[1] Several Glu transporters have also been shown to play a crucial role in the modulation of neurotransmission.[2] Glu is involved in most brain functions such as learning, memory, motor control, vision and pain sensitivity. The development of selective ligands for GluRs allows a fine modulation of the glutamatergic pathways and may provide an appropriate therapeutic approach for the treatment of neurodegenerative pathologies or neuropsychiatric disorders.[1] Glu is a highly flexible molecule and conformationally constrained analogues have been designed and synthesized in order to elucidate the conformational requirements for binding at individual receptors. For this purpose, alicyclic Glu analogues are of particular interest, and intensive studies have been carried out on three- to seven-membered ring analogues.[3–5] Among them, *trans*-(2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (L-CCG-I) has been shown to be a potent agonist of group II mGluRs while the *cis*-(2*S*,1′*S*,2′*R*)-isomer (L-CCG-III) is an inhibitor of glutamate transport systems at the excitatory synapses (Fig. 1).[4] In contrast, very limited work has been done on 2-(2′-carboxycyclobutyl)glycine (CBG) and to our knowledge only two of the four stereoisomers of the L-series, L-CBG-I and L-CBG-III, have been synthesized.[5]

Over the last few years, we have been developing a chemoenzymatic approach for the synthesis of Glu derivatives, based on the stereoselective aminotransfer-

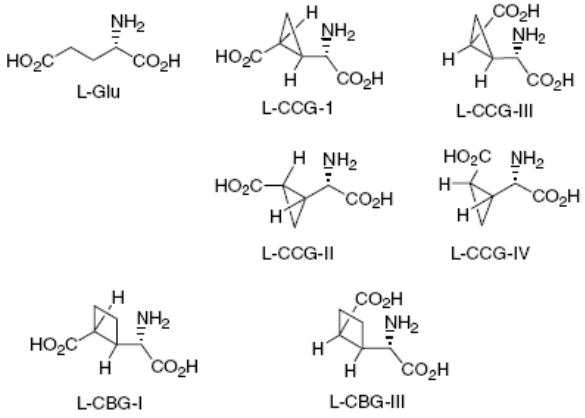


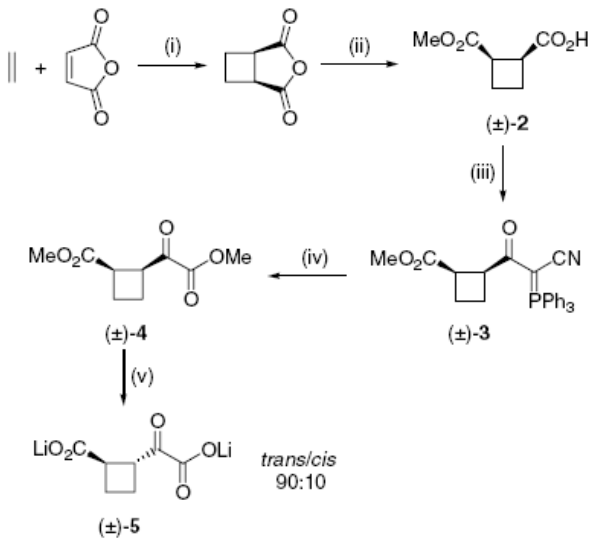
Figure 1. L-glutamic acid and alicyclic analogues.

* Corresponding authors. Tel.: +33 4 73 40 74 81; fax: +33 4 73 40 77 17 (D.J.A.); tel.: +33 4 73 40 71 28; fax: +33 4 73 40 77 17 (T.G.); e-mail addresses: David.AITKEN@univ-bpclermont.fr; Thierry.GEFFLAUT@univ-bpclermont.fr

ase-catalyzed conversion of α-keto acids to L-amino acids. We have shown that aspartate aminotransferase (AAT, EC 2.6.1.1) is a useful tool for the stereospecific synthesis of a variety of 4-substituted Glu derivatives from the corresponding 2-ketoglutaric acid (KG) analogues, easily prepared in racemic form.[6] The extension of the method to 3,4-disubstituted derivatives such as CBG was a stimulating challenge. The present work describes the original synthesis of the two *trans* isomers of 2-(2′-carboxycyclobutyl)glycines (CBG) by a highly selective enzymatic transamination of the appropriate cyclobutane derivative of α-ketoglutaric acid using one of two aminotransferases from *Escherichia coli*: aspartate aminotransferase (AAT) and branched chain aminotransferase (BCAT).

2. Synthesis of the *trans*-cyclobutane analogue of KG

The four-membered ring derivative of α-ketoglutaric acid was efficiently synthesized in racemic form as follows (Scheme 1): a [2+2] photocycloaddition reaction between maleic anhydride and ethylene was performed in acetonitrile solution in the presence of acetophenone as photosensitizer by irradiation for 5 h with a 400 W medium-pressure mercury lamp fitted with a pyrex filter. *cis*-1,2-Cyclobutane dicarboxylic anhydride **1** was isolated in 75% yield.[7] Methanolysis of this anhydride moiety gave the monoester *cis*-2-(methoxycarbonyl)cyclobutanecarboxylic acid **2**. Homologation of **2** into an α-ketoester was performed using Wasserman's methodology:[8] the acid function of **2** was coupled with (triphenylphosphoranylidene)acetonitrile to yield the α-keto cyanophosphorane **3** in 91% yield. Ozonolysis of intermediate **3** in a 3:1 mixture of dichloromethane/methanol at −78 °C provided *cis*-2-oxalylcyclobutanecarboxylic dimethyl ester **4** in 81% yield. Final ester hydrolysis

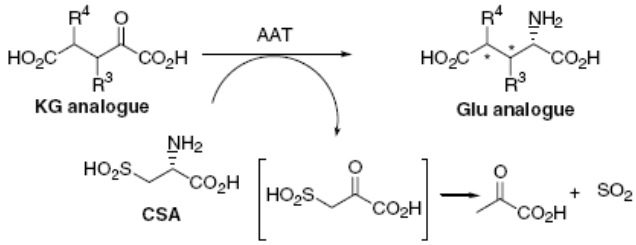


Scheme 1. Reagents and conditions: (i) acetophenone, CH_3CN, *hν*, pyrex, 5 h, 75%; (ii) MeOH, reflux, 16 h, 77%; (iii) (triphenylphosphoranylidene)-acetonitrile, EDCI, DMAP, CH_2Cl_2, rt, 4 h, 91%; (iv) O_3, CH_2Cl_2/MeOH 3:1, −78 °C, 81%; (v) LiOH, MeOH, rt, 2 h then Dowex resin H^+, 100%.

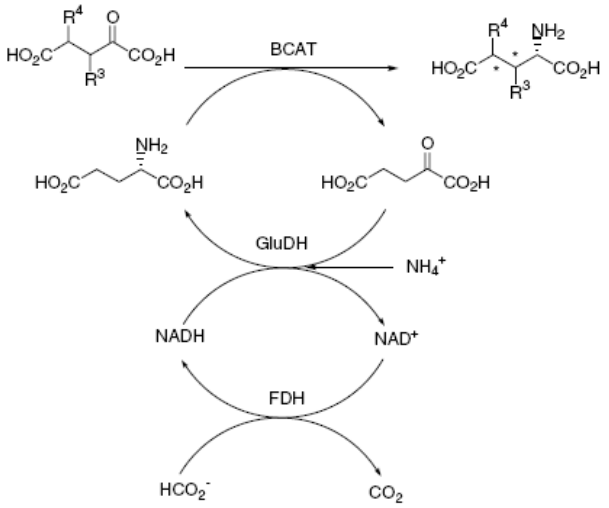
was carried out in the presence of a stoichiometric amount of lithium hydroxide in MeOH. In these reaction conditions, regiospecific epimerization α ketone functionality was observed, furnishing the thermodynamically more stable *trans* cyclobutane. The lithium salt **5** was isolated as a 90:10 *trans*/*cis* mixture determined by ^1H NMR. The transamination substrate **5** was thus prepared in five steps with an overall yield of 42% from maleic anhydride.

3. Enzymatic transaminations

E. coli Aspartate aminotransferase (AAT) catalyzes the equilibrium between Glu + oxalacetate on one side and KG + aspartic acid (Asp) on the other side. This enzyme presents a broad substrate spectrum towards 4-substituted KG analogues and shows marked enantioselectivity in favour of the 4*R* configuration of 4-alkyl derivatives.[6c–e,9] Kinetic resolution of racemic 4-alkyl KGs thus allows the preparation of (2*S*,4*R*)-4-alkyl Glu with very high ee and de. However, the enzyme specificity towards 3-substituted Glu is restricted to the (3*R*)-3-methyl derivative (data not published). *E. coli* Branched chain aminotransferase (BCAT) is a broader spectrum aminotransferase since it is involved in the metabolism of Glu, Val, Leu, Ile, Phe and Met.[10] Several examples of BCAT-catalyzed syntheses of unnatural amino acids, such as L-*tert*-leucine and L-phosphinothricine have already been described.[11] Enzymatic transamination is a useful approach to the synthesis of amino acids, provided that an equilibrium shift is performed.[12] AAT offers the opportunity of decarboxylation of oxalacetate, formed when Asp is used as the amino donor substrate. This decarboxylation can be catalyzed chemically or by enzymatic means and generates pyruvate which is not a substrate for AAT. Alternatively, a close analogue of Asp, cysteine sulfinic acid (CSA), can be used as the amino donor and furnishes an even more unstable β-keto sulfinic acid, which is readily decomposed into pyruvate and sulfur dioxide without the need for catalysis (Scheme 2).[13] Moreover, because of the high acidity of CSA, the purification of the amino acid product by ion exchange chromatography is highly simplified. Unfortunately, Asp or CSA are not substrates for BCAT and an alternative strategy has to be used for the equilibrium shift. This can be achieved through the enzymatic regeneration of the amino donor Glu used in a catalytic amount. Glutamic dehydrogenase (EC 1.4.1.3) can thus be used to catalyze the conversion of KG back to Glu in the presence of



Scheme 2. Aspartate aminotransferase-catalyzed transamination.

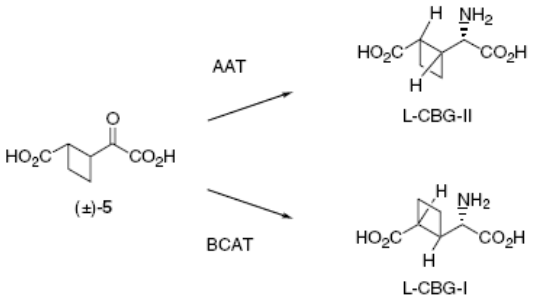


Scheme 3. Branched chain aminotransferase-catalyzed transamination.

ammonium ions and of the reduced coenzyme NADH. The coenzyme is also used in catalytic amount and is in turn regenerated using a stoichiometric amount of formic acid and formate dehydrogenase (EC 1.2.1.2). This last irreversible step affords the overall equilibrium shift (Scheme 3).

Since the cyclobutane KG analogue **5** was isolated as a mixture of *cis* and *trans* stereomers, we could not determine reliable kinetic parameters for the *trans* derivative. However, analytical measurements allowed us to evidence that sample (+/−)-**5** was a substrate for *E. coli* AAT and BCAT with, for each enzyme, a relative activity of around 2% of that for the natural substrate KG. As these aminotransferases are characterized by high specific activities, this activity proved to be sufficient for synthetic purposes.

With *E. coli* AAT, the synthesis was carried out in water (140 mL) at pH adjusted to 7.6 with α-keto carboxylate **5** (*cis*/*trans* 10:90) (2.8 mmol) in the presence of a stoichiometric amount of CSA as the amino donor and aminotransferase (5 mg, 250 UI). Acetaldehyde (2.8 mmol) was added to the reaction mixture to scavenge sulfite ions produced from CSA and thus increase the reaction rate by preventing partial inhibition of the enzyme by this product. The conversion rate was determined by titration of pyruvate formed from CSA using the couple lactic dehydrogenase (LDH, EC 1.1.1.27) and NADH: NADH consumed for pyruvate reduction was titrated in small aliquots by absorbance measurements at 340 nm. After 4 h, near 45% conversion, the reaction slowed markedly and was stopped by passing the reaction mixture directly through a short column of Dowex 50 resin (H^+ form). Unreacted α-ketoacid, pyruvate and excess CSA were eluted simply with water and the adsorbed glutamic acid analogue was further eluted with a 1 M ammonium hydroxide solution. The Glu analogue was further purified by adsorption onto a short column of strongly basic Dowex 2 resin (AcO^- form) followed by elution with an AcOH gradient (0 → 1 M). CBG-II was thus isolated as a white solid in 32% yield with a very good purity (Scheme 4).[15]

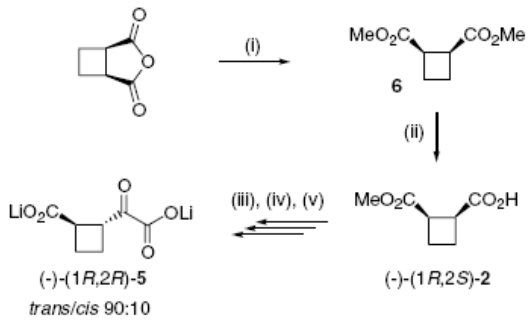


Scheme 4. Enzymatic transamination of *trans*-2-oxalylcyclobutane carboxylic acid.

Transamination with BCAT was achieved on substrate **5** (0.3 mmol) with a catalytic amount of Glu (0.06 mmol) as the amino donor and *E. coli* BCAT (0.5 mg, 4 UI). The regeneration system comprised NADH (0.03 mmol), ammonium formate (0.6 mmol), pig liver glutamic dehydrogenase (0.4 mg, 9 UI), yeast formate dehydrogenase (2 mg, 12 UI). The reaction was followed by TLC during 48 h. The cyclobutane Glu analogue was purified by ion exchange chromatography as previously described and isolated as a white solid in 26% yield (Scheme 4).[16]

Absolute configurations were attributed as follows. Physicochemical and spectroscopic data for the isomer obtained with BCAT were comparable with those for the *trans* isomer already described in the literature.[5] It was shown to be (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclobutyl)glycine L-CBG-I with optical rotation $[\alpha]_D^{20}$ +96.4 (*c* 0.7, H_2O) [lit. $[\alpha]_D^{20}$ +97.1 (*c* 0.49, H_2O)] and mp over 245 °C (dec.) [lit. mp 258–263 (dec.)] (Scheme 4). The isomer obtained with AAT displayed different spectral data and had $[\alpha]_D^{25}$ −53.0 (*c* 0.7, H_2O) and mp 164–166 °C. Owing to AAT selectivity for L-amino acids, the (2*S*,1′*R*,2′*R*) configuration was assigned to this second *trans* isomer L-CBG-II (Scheme 4). To our knowledge, this compound has not been described before. In order to confirm this stereochemistry, enantiomerically pure (1*R*,2*R*)-2-oxalylcyclobutane carboxylic acid **5** was prepared as described in Scheme 5 and evaluated as a substrate for AAT. The synthesis involved the sequence already described for racemic compound **5** from enantiomerically pure *cis*-(1*R*,2*S*)-**2**. This compound was prepared by desymmetrization of *meso*-diester **6** by pig liver esterase-catalyzed hydrolysis as described in the literature.[14] Analytical rate measurement of AAT-catalyzed transamination of (−)-(1*R*,2*R*)-**5** gave relative activity very close from that measured with racemic compound **5**. (−)-(1*R*,2*R*)-**5** is thus the enantiomer recognized by the enzyme confirming our configuration assignment for L-CBG-II.

In conclusion, aminotransferases AAT and BCAT present complementary enantioselectivities towards *trans*-2-oxalylcyclobutanecarboxylic acid **5**, allowing selective access to isomeric (2*S*,1′*R*,2′*R*)- and (2*S*,1′*S*,2′*S*)-CBGs.



Scheme 5. Reagents and conditions: (i) MeOH, c. H_2SO_4, reflux, 4 h, 60% (ii) PLE, pH 7, 20 °C, 78%; (iii) (triphenylphosphoranylidene)acetonitrile, EDCI, DMAP, CH_2Cl_2, rt, 4 h, 85%; (iv) O_3, CH_2Cl_2/MeOH 3:1, −78 °C, 1 h 30 (v) LiOH, MeOH then Dowex resin H^+, 2 h, 87% (two steps).

Biological activities of these compounds towards glutamate receptors and transporters are under investigation. This study shows the potential of AAT and BCAT for chemoenzymatic synthesis of alicyclic analogues of L-glutamic acid, a goal which we are currently pursuing.

Acknowledgements

We are grateful to the CNRS for financial support. We would like to thank Pr. Kagamiyama's group (Osaka Medical College) for providing us with *E. coli* strains which over express AAT and BCAT.

References and notes

1. (a) Blasi, A. D.; Conn, P. J.; Pin, J. P.; Nicoletti, F. *Trends Pharmacol. Sci.* **2001**, *22*, 114–120; (b) Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, E.; Madsen, U.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2000**, *43*, 2609–2645; (c) Watkins, J. C. *Biochem. Soc. Trans.* **2000**, *28*, 297–310; (d) Schoepp, D. D.; Jane, D. E.; Monn, J. A. *Neuropharmacology* **1999**, *38*, 1431–1476; (e) Pin, J. P.; De Colle, C.; Bessis, A. S.; Acher, F. *Eur. J. Pharm.* **1999**, *375*, 277–294.
2. (a) Shigeri, Y.; Seal, R. P.; Shimamoto, K. *Brain Res. Rev.* **2004**, *45*, 250–265; (b) Amara, S. G.; Fontana, A. C. K. *Neurochem. Int.* **2002**, *41*, 313–318; (c) Danbolt, N. C. *Prog. Neurobiol.* **2001**, *65*, 1–105.
3. (a) Collado, I.; Pedregal, C.; Bueno, A. B.; Marcos, A.; Gonzalez, R.; Blanco-Urgoiti, J.; Perez-Castells, J.; Schoepp, D. D.; Wright, R. A.; Johnson, B. G.; Kingston, A. E.; Moher, E. D.; Hoard, D. W.; Griffey, K. I.; Tizzano, J. P. *J. Med. Chem.* **2004**, *47*, 456–466; (b) Nakazato, A.; Sagakami, K.; Yasuhara, A.; Ohta, H.; Yoshikawa, R.; Itoh, M.; Nakamura, M.; Chaki, S. *J. Med. Chem.* **2004**, *47*, 4570–4587; (c) Conti, P.; De Amici, M.; Roda, G.; Vistoli, G.; Stensbøl, T. B.; Bräuner-Osborne, H.; Madsen, U.; Toma, L.; De Micheli, C. *Tetrahedron* **2003**, *59*, 1443–1452; (d) Bunch, L.; Liljefors, T.; Greenwood, J. R.; Frydenvang, K.; Bräuner-Osborne, H.; Krogsgaard-Larsen, P.; Madsen, U. *J. Org. Chem.* **2003**, *68*, 1489–1495; (e) Pellicciari, R.; Marinozzi, M.; Camaioni, E.; Nùnez, M. C.; Costantino, G.; Gasparini, F.; Giorgi, G.; Macchiarulo, A.; Subramanian, N. *J. Org. Chem.* **2002**, *67*, 5497–5507; (f) Costantino, G.; Macchiarulo, A.; Pellicciari, R. *J. Med. Chem.* **1999**, *42*, 2816–2827; (g) Acher, F. C.; Tellier, F. J.; Azerad, R.; Brabet, I. N.; Fagni, L.; Pin, J. P. *J. Med. Chem.* **1997**, *40*, 3119–3129; (h) Monn, J. A.; Valli, M. J.; Massey, S. M.; Wright, R. A.; Salhoff, C. R.; Johnson, B. G.; Howe, T.; Alt, C. A.; Rhodes, G. A.; Robey, R. L.; Griffey, K. R.; Tizzano, J. P.; Kallman, M. J.; Helton, D. R.; Schoepp, D. D. *J. Med. Chem.* **1997**, *40*, 528–537.
4. (a) Chavan, S. P.; Sharma, P.; Sivappa, R.; Bhadbhade, M. M.; Gonnade, R. J.; Kalkote, U. R. *J. Org. Chem.* **2003**, *68*, 6817–6819; (b) Rifé, J.; Ortuño, R. M. *J. Org. Chem.* **1999**, *64*, 8958–8961; (c) Shimamoto, K.; Ohfune, Y. *J. Med. Chem.* **1996**, *39*, 407–423; (d) Shimamoto, K.; Ishida, M.; Shinozaki, H.; Ohfune, Y. *J. Org. Chem.* **1991**, *56*, 4167–4176.
5. (a) Tsujishima, H.; Nakatani, K.; Shimamoto, K.; Shigeri, Y.; Yumoto, N.; Ohfune, Y. *Tetrahedron Lett.* **1998**, *39*, 1193–1196; (b) Pohlman, M.; Kazmaier, U. *Org. Lett.* **2003**, *5*, 2631–2633.
6. (a) Hélaine, V.; Rossi, J.; Gefflaut, T.; Alaux, S.; Bolte, J. *Adv. Synth. Catal.* **2001**, *343*, 692–697; (b) Bessis, A. S.; Bolte, J.; Pin, J. P.; Acher, F. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1569–1572; (c) Hélaine, V.; Rossi, J.; Bolte, J. *Tetrahedron Lett.* **1999**, 6577–6580; (d) Hélaine, V.; Bolte, J. *Eur. J. Org. Chem.* **1999**, 3403–3406; (e) Echalier, F.; Constant, O.; Bolte, J. *J. Org. Chem.* **1993**, *58*, 2747–2750.
7. The photocycloaddition procedure is a modification of the method described by Owsley, D. C.; Bloomfield, J. J. *J. Org. Chem.* **1971**, *36*, 3768–3773.
8. Wasserman, H. H.; Ho, W.-B. *J. Org. Chem.* **1994**, *59*, 4364–4366.
9. *E. coli* AAT was produced and purified following a described procedure from an overexpressing *E. coli* strain TY103 transformed with pUC19-*aspC*: Kamitori, S.; Hirotsu, K.; Higuchi, T.; Kondo, K.; Inoue, K.; Kuramitsu, S.; Kagamiyama, H.; Higuchi, Y.; Yasuoka, N.; Kusunoki, M.; Matsuura, Y. *J. Biochem.* **1987**, *101*, 813–816.
10. *E. coli* BCAT was produced and purified following a described procedure from an overexpressing *E. coli* strain TY103 transformed with pUC19-*IlvE*: Inoue, K.; Kuramitsu, S.; Aki, K.; Watanabe, Y.; Takagi, T.; Nishigai, M.; Ikai, A.; Kagamiyama, H. *J. Biochem.* **1988**, *104*, 777–784.
11. (a) Li, T.; Kootstra, A. B.; Fotheringham, I. G. *Org. Proc. Res. Dev.* **2002**, *6*, 533–538; (b) Then, J.; Bartsch, K.; Deger, H. M.; Grabley, S.; Marquardt, R. U.S. Patent 5,962,281, 1999.
12. (a) Stewart, J. D. *Curr. Opin. Chem. Biol.* **2001**, *5*, 120–129; (b) Ager, D. J.; Li, T.; Pantaleone, D. P.; Senkpeil, R. F.; Taylor, P. P.; Fotheringham, I. G. *J. Mol. Catal. B: Enzymatic* **2001**, *11*, 199–205; (c) Ager, D. J.; Fotheringham, I. G.; Li, T.; Pantaleone, D. P. *Enantiomer* **2000**, *5*, 235–243; (d) Taylor, P. P.; Pantaleone, D. P.; Senkpeil, R. F.; Fotheringham, I. G. *TIBTECH* **1998**, *16*, 412–418.
13. Jenkins, W. T.; D'Ari, L. *Biochem. Biophys. Res. Commun.* **1966**, *22*, 376–382.
14. Sabbioni, G.; Jones, J. B. *J. Org. Chem.* **1987**, *52*, 4565–4570.
15. L-CBG-II: white solid; mp 164–166 °C; $[\alpha]_D^{20}$ −53.0 (*c* 0.7, H_2O); ^1H NMR (400 MHz, D_2O) δ 1.79–2.13 (m, 4H), 2.86 (dq, $J = 8.0$, 9.2 Hz, 1H), 3.07 (q, $J = 9.2$ Hz, 1H), 3.68 (d, $J = 7.6$ Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 21.0 (CH_2), 21.6 (CH_2), 38.0 (CH), 41.3 (CH), 57.4 (CH), 172.8 (C), 178.5 (C).
16. L-CBG-I : white solid ; mp > 245 °C (dec.); $[\alpha]_D^{20}$ +96.4 (*c* 0.7, H_2O); ^1H NMR (400 MHz, D_2O) δ 1.79–1.95 (m, 2H), 1.96–2.11 (m, 2H), 2.78 (dq, $J = 8.8$, 9.2 Hz, 1H), 3.15 (q, $J = 9.2$ Hz, 1H), 3.67 (d, $J = 8.8$ Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 20.9 (CH_2), 21.4 (CH_2), 38.9 (CH), 41.7 (CH), 57.8 (CH), 172.6 (C), 178.5 (C).